ATTACHMENT A

UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Chang et al.	Art Unit	1633
Serial No:	10/712,359	Examiner	K. Hiriyanna
Filed:	November 13, 2003	Conf. No.	1306
For:	DOMINANT NEGATIVE VARIANTS OF METHIONINE AMINOPEPTIDASE 2 (METAP2) AND CLINICAL USES THEREOF		

DECLARATION OF DR. YIE-HWA CHANG UNDER 37 C.F.R. §1.132

I, Yie-Hwa Chang, declare and state as follows:

1. I have over twenty-five years of experience in the fields of chemistry and molecular biology. I am currently employed as a tenured associate professor for St. Louis University, and have worked at St. Louis University since 1991. Additionally, I am the Founder and President of Mediomics, LLC, a company specializing in developing and selling molecular biology assays. My educational background includes a Bachelor of Science degree in Chemistry awarded by the National Taiwan University in 1977 and a doctorate degree (i.e., PhD) in Chemical Biology awarded by the California Institute of Technology in 1986. I have also published over twenty-five scientific papers and presented numerous abstracts at internationally attended meetings. Attached to this declaration is a copy of my curricula vitae.

2. I am a co-inventor of U.S. Patent Application Publication No. 2005/0032221 ('221 application) entitled "Dominant Negative Variants of Methionine Aminopeptidase 2 (MetAP2) and Clinical Uses Thereof." In light of my first hand knowledge of the '221 application and my knowledge of the state of the art at the time of the filing of the application, I state the following:

    a. Based on my experience, it is my considered belief that it was known in the art at the time the '221 application was filed that inhibition of MetAP2 with compounds such as fumagillin, ovalicin, and TNP-470 resulted in decreased cell proliferation. Further, one skilled in the art would know that these compounds were used to inhibit MetAP2 in several different tumor studies, both *in vitro* and *in vivo*, including Kaposi's sarcoma, renal cell carcinoma, brain

cancer, breast cancer, cervical cancer and prostate cancer, and therefore, the inhibition of MetAP2 was known to those skilled in the art to modulate the cell proliferation of multiple different cell types.

b. Furthermore, *in vitro* studies using Human Vascular Endothelial (HUVE) cells are recognized in the art as a model system for studying cell proliferation, and studies using HUVE cells are recognized in the art as correlating with *in vivo* events. For instance, early testing on compounds such as TNP-470, were performed in HUVE cells. See, for example, Br J Cancer (1994) 69(2):212-16 and J Pharmacol Toxicol Methods (2000) 43(1):15-24.

c. One of skill in the art would be able to follow the teachings of the specification to create a MetAP2 variant that lacks aminopeptidase activity but comprises a translation domain, and thereby create a MetAP2 variant that possesses dominant negative activity. Additionally, one of skill in the art, In light of the specification, would be able to engineer a polynucleotide to express a MetAP2 variant with dominant negative activity.

d. A skilled researcher in the art knows that a catalytically inactive variant is not synonymous with a dominant negative variant, and that it would not be obvious, likely, or intuitive that a catalytically inactive variant would possess dominant negative activity. Stated another way, dominant negative activity does not necessarily and inevitably flow from a catalytically inactive variant.

e. Yeast, a single-cell organism, is a simple eukaryote. In contrast, humans are a highly complex multi-cellular eukaryote. These two organisms represent the spectrum of eukaryotic complexity. Hence, a skilled researcher would reasonably conclude that if a dominant negative variant of MetAP2 inhibits cell proliferation in both a yeast cell and a human cell, a dominant negative variant of MetAp2 would also inhibit cell proliferation in a eukaryotic organism that falls between yeast and humans in complexity.

3. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Ye-Hwa Chang

June 11, 2007
Date

Curriculum Vitae

Yie-Hwa Chang, Ph.D.

Education:

B.S. - Chemistry (Organic Chemistry major), National Taiwan University (1972-1977);

Ph.D. - Chemistry (Chemical Biology major), California Institute of Technology (1980-1986);

Research Fellow - Department of Molecular Biology, Massachusetts General Hospital and Department of Genetics, Harvard Medical School (1986-1991).

Current Position and Address:

Associate Professor (Tenured)
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Saint Louis University School of Medicine
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Director
Proteomics Facility
Department of Biochemistry and Molecular Biology

Member
Liver Center
St. Louis University Medical School

Previous Professional Experience:

- Participant, CSHL "Protein Crystallography" course, Cold Spring Harbor Laboratory (1990);
- Research Assistant (Biochemistry), Department of Chemistry, California Institute of Technology (1980-1986);
- Research Assistant (Organic Chemistry), Department of Chemistry, National Taiwan University (1979-198.Q).

Professional Society Memberships:

- American Society of Biochemistry and Molecular Biology (1989-present);
- American Chemical Society (1989-present);
- Society of Chinese Bioscientists in America (1985-present)

Awards and Hornors:

Mallinckrodt Young Faculty Award
(1991-1994)

Professional Services: (University or Departmental)

- Chemical Society, Chairman, National Taiwan University (1976);
- Chinese Student Association, Chairman, California Institute of Technology (1982);
- Chinese Student Association of Southern California} Vice President (1983);
- Faculty Recruiting Committee, St. Louis University School of Medicine (1991-1992);
- Graduate Student Recruiting Committee, St. Louis University School of Medicine (1992-present);
- Graduate Curriculum Committee, St. Louis University School of Medicine (1992-1997);
- Graduate Curriculum Committee, Chairman, St. Louis University School of Medicine (1996);
- Curriculum Committee for Faculty Development, St. Louis University School of Medicine (1996);
- Faculty Search Committee (1999).
- Graduate Curriculum Committee (2005- present)

Public Service:

- Chair, Biotechnology Session, Science and Technology Conference for Midwest Chinese American (1994, 1998);
- Chair of Scientific Program Committee, Science and Technology Conference for Midwest Chinese American (1995);
- Conference Chairman, Science and Technology Conference for Midwest Chinese American (1996, 1997);
- The Association of Science and Technology for Midwest Chinese American (Founder, 1999);
- St. Louis Academy of Sciences, Volunteer, (1995-present);
- Ad hoc reviewer for Proc. Natl. Acad. Sci., FASEB. J., J. Biol. Chem., etc.

Patents and Pending Patent Applications:

1.) U.S. Patent Application, Serial No: 08/595,025 (Filed 01/31/1996; Abandoned), titled "Clone of a Nucleotide Sequence Encoding a Protein Having Two Functions," Inventor: Yie-Hwa Chang.
2.) U.S. Patent No. 5,888,796 (Date: 03/30/1999), titled "Clone of a Nucleotide Sequence Encoding a Protein Having Two Functions," Inventor: Yie-Hwa Chang.
3) U.S. Patent Application, Serial No: 09/943,123 (Filed 08/30/2001), titled "Dominant Negative Variants of Methionine Aminopeptidase 2 (MetAP2) and

Clinical Uses Therefor," Co-Inventors: Yie-Hwa Chang, William S. Micka, and Joseph A. Vetro.

3.) U.S. Patent Application, Serial No. 09/864,732 (Filed 05/24/2001; Divisional of US Patent No. 6,621,794 B1), titled ""Methods for Identifying Inhibitors of Methionine Aminopeptidases," Inventor: Yie-Hwa Chang
4.) U.S. Patent Application, Serial No. 09/928,385 (Filed 08/13/2001), titled "A Rapid and Sensitive Proximity-Based Assay for the Detection and Quantification of DNA Binding Factors," Inventor: Tomasz Heyduk.
5.) European Patent Application, Nationalized PCT, Serial No. 00984526.4 (Filed: 04/18/2002), titled "Methods for Identifying Inhibitors of Methionine Aminopeptidases," Inventor: Yie-Hwa Chang.
6.) European Patent Application, Nationalized PCT, Serial No. 02761229.0 (Filed 03/18/2004), titled "Dominant Negative Variants of Methionine Aminopeptidase 2 (MetAP2) and Clinical Uses Therefor," Inventor: Yie-Hwa Chang.
7.) U.S. Patent Application, Serial No: 10/888,962 (Filed 07/09/2004), titled "Compositions and Methods for Inhibiting," Inventor Yie-Hwa Chang.
8.) Canada Patent Application, Nationalized PCT, Serial No: 2,387,126 (Filed 10/12/2000), titled "Methods for Identifying Inhibitors of Methionine Aminopeptidases," Inventor: Yie-Hwa Chang.
9.) Japan Patent Application, Nationalized PCT, Serial No: 2001-530447 (Filed 10/12/2000), titled "Methods for Identifying Inhibitors of Methionine Aminopeptidases," Inventor: Yie-Hwa Chang.
10) PCT Patent Application, Serial No: PCT/US02/24661 (Filed 08/02/2002), titled "Dominant Negative Variants of Methionine Aminopeptidase 2 (MetAP2) and Clinical Uses Thereof," Inventor: Yie-Hwa Chang.
11) U.S. Patent No. 5,888,796 (Date: 3/30/99), title "Clone of a Nucleotide Sequence Encoding a Protein Having Two Functions", Inventor: Yie-Hwa Chang.
12) U.S. Patent No. 5,885,820 (Date: 3/23/99), title "Clone of a Nucleotide Sequence Encoding a Protein Having Two Functions", Inventor: Yie-Hwa Chang.
13) US Patent No. 6, 261,794 B1 (Date: July 17, 2001), title "Methods for Identifying Inhibitors of Methionine Aminopeptidases", Inventor: Yie-Hwa Chang.
14) PCB' Patent Application, PC '/USOO/41146 (Filed: 10/12/01), title "Methods for Identifying Inhibitors of Methionine Aminopeptidases", Inventor: Yie-Hwa Chang.
15) US Patent Application, Serial No. 09/943,123 (Filed: 08/30/01), "Dominant Negative Variants of Methionine Aminopeptidase 2 (MetAP2) and Uses Therefor", Inventors: Yie-Hwa Chang, William S. Micka (student) and Joseph A. Vetro (student).

Fundings:

1. Mallinkarodt Foundation Award (PI, 1991-1994)
Title: Amino-terminal processing of proteins.
2. National Science Foundation (PI, 1994-1998)
Title: Mechanism of N-terminal protein processing
3. American Cancer Society (PI, 1998-2002)
Title: Role of MetAPs in angiogenesis
4. NIH (Co-PI, 2003-2006)
Title: Pepsin as a biomarker for aspiration
5. Liver Center (PI, 2005-2007)
Identification of novel biomarkers for HCC
6. NIH (Co-PI, 2006-2009)

Title: Pepsin as a biomarker for aspiration.

Current and Past Teaching Responsibilities:

- Molecular Biology and Genetics (case-based tutorials) (1998-present);
- Metabolism (Medical School Course) (1997-present);
- Preparation and Evaluation of Research Proposals, St. Louis University School of Medicine (1996);
- Advanced Topics for Biochemistry and Molecular biology, St. Louis University School of Medicine (1992, 1994, 2000);
- Biochemistry, St. Louis University School of Medicine (1991-present);
- Instructor of CSHL course of "Protein Purification & Characterization", Cold Spring Harbor Laboratory (1989);
- Teaching Assistant of Biochemistry, California Institute of Technology (1982-1984);.
- Teaching Assistant of Organic Chemistry, National Taiwan University (1979-1980);
- Thesis Dissertation, Postdoctoral Supervision.

Graduate Students Supervised:

- Ben Dummitt (2000-2005);
- Joe Vetro, Ph.D. student (1997-2001);
- Lashonda Williams, M.D. (2001);
- Marco Klinkenberg, Ph.D. student (1992-1998);
- William S. Micka, Medical School Student (1999-present);
- Scott Turner, Medical School Student (1999-present);
- Songlan Zuo, Ph.D. student (1991-1995);
- Xuan Li, Ph.D. student (1993-1996);
- Grace Wang, summer student (1996) (University of Pennsylvania);
- Arpitha Reddy, Doisy summer program, MD student (1991, 1993);
- Rajiv R. Doshi, Doisy summer student (1994);
- Buu-Chau T. Do, undergraduate student (1993 -1996);
- Anthony Zimmerman, undergraduate student (1991-1993);
- Noel Baichoo (Thesis committee);
- Tao Zhao (Thesis committee);
- Sandhya Callaci (Thesis committee).
- Leonard B. Maggi (Thesis Committee)

Postdoctoral Fellows Supervised:

- Shurong Xue, Ph.D. Postdoctoral Fellow (2000 -2001);
- Shaoping Chen, M.D. (1995-1999);
- Aftab Alam, Ph.D. (1991-1993).

Publications:

1. Chang YH, Teichert U, Smith JA. Purification and characterization of methionine-specific aminopeptidase from *Saccharomyces cerevisiae*. J. Biol Chem. 1990, 265:19892-19897.

2. Chang YH, Labgold MR, Richards JH. Altering enzymatic activity. Recruitment of carboxypeptidase activity into an RTEM- 1 13-lactamase/penicillin-binding-protein 5 chimera. Proc. Natl. Acad. Sci. U.S.A. 1990; 87:2823-2827.
3. Liu Z, Williams KP, Chang YH, Smith JA. Single amino acid substitution alters T cell determinant selection during antigen processing. J. Immunol. 1991; 146:438-443.
4. Chang YH, Teichert U, Smith JA. Molecular cloning, sequencing, deletion and overexpression of a eukaryotic methionine aminopeptidase gene from Saccharomyces cerevisiae. J. Biol. Chem. 1992; 267:8007-8011.
5. Liu Z, Williams KP, Chang YH, Smith JA. Immunodominance : A single amino acid substitution within an antigenic site alters intramolecular selection of T cell determinants J. Immunol.1993; 151:1-7.
6. Zuo SL, Guo Q, Chang YH. A protease assay via pre-column derivatization and high pressure liquid chromatography. Analytical Biochemistry 1994, 222:514-516.
7. Zuo SL, Guo Q, Chang YH. Evidence that zinc fingers in a methionine aminopeptidase from S. cerevisiae are important for normal growth. Mol. Gen. Genetics 1995, 246:247-253.
8. Li X, Chang YH. Cloning of a human cDNA encodes a protein associated with initiation factor-2. Biochim, Biophys. Acta. 1995, 1260:333-336.
9. Li X, Chang YH. Amino-terminal protein processing in *Saccharomyces cerevisiae* is an essential function that requires two distinct methionine aminopeptidases. Proc. Natl. Sci. Acad. USA 1995, 92:12357-12361.
10. Li X, Chang YH. Evidence that the human homologue of a rat initiation factor-2 associated protein (p67) is a methionine aminopeptidase. Biochem Biophys. Res. Commun. 1996, 227:152-159.
11. Griffith EC, Su Z, Turk B, Chen S, Chang YH, Wu Z, Biemann K, Liu JO. Methionine aminopeptidase (Type 2) is the common target for angiogenesis inhibitors AGM-1470 and ovalicin. Chemistry and Biology, 1997, 4:461-471.
12. Klinkenberg M, Ling C, Chang YH. A dominant negative mutation in *S. cerevisiae* methionine aminopeptidase-1 affects catalysis and interferes with the function of methionine aminopeptidase-2. Arch. Biochem. Biophys. 1997, 347:193-200.
13. Griffith EC, Su, Z, Niwayama S, Ramsay CA, Chang YH. Molecular recognition of angiogenesis inhibitors fumagillin and ovalicin by methionine aminopeptidase 2. Proc. Natl. Acad. Sci. USA 1998, 95:15183-15188.
14. Turk BE, Griffith EC, Wolf S, Bieman K, Chang YH, Liu JO. Selective inhibition of N-terminal processing by TNP-470 and Ovalicin in endothelial cells. Chemistry and Biology,1999, 6:823-833.
15. Udagawa T, Yuan J, Panigrahy D, Chang YH, Shah J, D'Amato RJ. Cytochalasin E, an epoxide containing Aspergillus-derived fungal metabolite, inhibits angiogenesis and tumor growth. J. Pharmacol. Exp. Ther. 2000, 294:421-427.
16. Kwon JY, Jeong HW, Kim HK, Kang KH, Chang YH, Bae KS, Choi JD, Lee UC, Son KH, Kwon BM cis-Fumagillin, a new methionine aminopeptidase (type 2) inhibitor produced by Penicillium sp. F2757. J. Antibiotics, 2000, 53:799-80.
17. Metheny N., Chang YH etc. Pepsin immunoassay as a marker for pulmonary aspiration. American J. Critical care, 2002, 11:150-154.

18. Son KH, Kwon JY, Joeng HW, Kim HK, Kim CJ, Chang YH, Choi JD, Kwon BM 5-Demethylovalicin, as a methionine aminopeptidase-2 inhibitor produced by Chrysoporium. Bioorganic & Medicinal Chemistry 2002, 10:185-188.
19. Chen S, Vetro J, Chang YH. The specificity *in vivo* of two distinct methionine aminopeptidase from *S. cerevisiae*. Arch Biochem. Biophys. 2002, 398:87-93.
20. Vetro J. and Chang YH. Yeast MetAP1 is ribosome-associated and requires its N-terminal zinc finger domain for normal function *in vivo*. J. Cell. Biochem. 2002, 85:678-688.
21. Dummitt B, Fei Y, Chang YH. Functional expression of human methionine aminopeptidase-1. Protein and Peptide Letters. 2002, 9:295-303.
22. Dummitt B., Micka W., Chang YH. N-terminal methionine removal and methionine metabolism in S. cerevisiae. J Cell Biochem. 2003 ,89:964-974.
23. Metheny NA, Dahms TE, Chang YH, Steward BJ, Frank PA, Clouse RE. Detection of pepsin in tracheal secretions after forced small aspirations of gastric juice. JPEN. 2004, 28:79-84.
24. Vetro J., Micka WS, Dummitt B, Chang YH. Identification and characterization of a dominant negative mutant of yeast methionine aminopeptidase 2. J Cell Biochem 2005, 94:656-658.
25. Babbitt SE, Kiss A, Deffenbaugh AE, Chang YH, Bailly E, Erdjument-Bromage H, Tempst P, Buranda T, Sklar LA, Baumler J, Gogol E, Skowyra D. ATP hydrolysis-dependent disassembly of the 26S proteasome is part of the catalytic cycle. Cell, 2005, 121:553-565.
26. Dummitt B, Micka WS, Chang YH Yeast Glutamine-fructose-6-phosphate Amidotransferase (Gfa1) requires methionine aminopeptidase activity for proper function J. Biol. Chem., 2005, 280:14356-14360.
27. Basu A, Saito K, Meyer K, Ray RB , Chang YH and Ray R. Secretory gelsolin fragments from hepatocytes induce stellate cell cytotoxicity. Apoptosis 2006 (in press)

Book Chapters:

1. Labgold MR, Chang YH, Richards JH. Catalysis by chimeric proteins: conversion of a β-lactamase to a D,D-carboxypeptidase.. Current Research in Protein Chemistry pp489-497. Edited by: Academic Press, Inc. 1990.
2. Liu Z, Chang YH, Williams KP, Kassel DB, Poellinger B, Smith JA. Single amino acid substitution alters T cell determinant selection during antigen processing of staphylococcal nuclease. Peptides pp. 895-897. Edited by Girat E and Andrew D: Leiden NV: Escom Science Publishers. 1990.
3. Chang YH. Biochemical and genetic analysis of yeast aminopeptidases. In Aminopeptidases. Edited by Allen Taylor: R.G. Landes Bioscience Publishers. 1996.
4. Chang YH. Methionine aminopeptidase, Encyclopedia of Molecular Medicine. Edited by Thomas E. Creighton. John Wiley & Sons, Inc. New York, N. Y. 2001.
5. Vetro J, Dummitt B & Chang YH, Angiogenesis: Emerging role of methionine aminopeptidases, Edited by Nigel Hooper, Kluwer Academic Publishers, 2004. (Vetro and Dummitt were Chang's graduate students).